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PERSPECTIVES IN BASIC SCIENCE

Gene targeting: Applications in transplantation research

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Gene targeting: Applications in transplantation research. Gene targeting, the manipulation of gene in the mouse genome using homologous recombination in embryonic stem cells, is a powerful experimental tool that has been widely utilized in a number of disciplines. The ability to precisely alter genes in this way provides an avenue for investigating the role of a gene product in normal and pathological processes in the intact animal, with a precision and efficacy not possible using pharmacological agents, antibodies or engineered proteins. In transplant research, gene targeting provides a unique tool for discriminating the contributions of gene expression in donor versus recipient tissues. This review focuses on several areas in transplantation research where gene targeting has made useful contributions. These include studies of the role of donor and recipient multiple histocompatibility complex antigens in regulating rejection responses, the role of $CD4^+$ T cell in mediating acute rejection, and the functions of cytokines during rejection and tolerance induction. These studies highlight the unique advantages of gene targeting in studies of complex processes in whole animals and illustrate the contributions of this technique to understanding the pathogenesis of allograft rejection.

Manipulation of genes in the mouse genome using homologous recombination in embryonic stem cells ("gene targeting") is a powerful experimental tool that has achieved widespread application in a number of scientific disciplines [1, 2]. Using this technique, alterations of cloned DNA produced in a test tube are inserted at precise locations in the mouse genome, where they are transmitted to subsequent generations as stable genetic traits. In the majority of mouse lines that have been produced using these techniques, null mutations are created in the targeted gene locus. These targeted gene disruptions are termed "gene knockouts." Although a gene knockout is the simplest alteration that can be produced through gene targeting, other more complex changes in the target gene can be accomplished, including the manipulation of individual nucleotides [3], duplication of the target locus with its associated regulatory regions [4], and alteration in the gene in only specific tissues [5].

The ability to alter genes precisely in this way has had immense utility in a number of areas. In particular, these technologies have provided an avenue for applying the power of molecular genetics to studies in whole animal systems. Thus, knockout mice can be used to determine the role of a gene product in normal and pathological processes in intact animals with a precision and efficacy that is not possible using pharmacological agents, antibodies, or engineered proteins. These techniques may be especially useful for identifying the role of an individual gene product in complex responses *in vivo*, such as the reaction that occurs when foreign tissue is transplanted into a genetically disparate host [6, 7]. Moreover, in transplantation research, gene targeting provides a unique tool for separately identifying the contribution of gene expression in donor versus recipient tissues by using the genetically manipulated animal as either the transplant donor or recipient.

Because of these characteristics, there has been wide ranging use of gene targeting in transplantation research to explore a variety of issues [8]. In this article, we review some of these applications while attempting to highlight unique contributions of gene targeting to these experiments. In some cases, knockout mice have confirmed existing paradigms, whereas in other cases, they have raised interesting new questions about the pathogenesis of allograft rejection.

GENE TARGETING STUDIES IN TRANSPLANTATION

Transplantation of organs in which expression of major histocompatibility complex antigens has been genetically altered

The rejection of transplanted tissue is triggered through the recognition of major histocompatibility complex (MHC) antigens (Fig. 1) from the donor by T cells from the recipient [reviewed in 7]. This allorecognition

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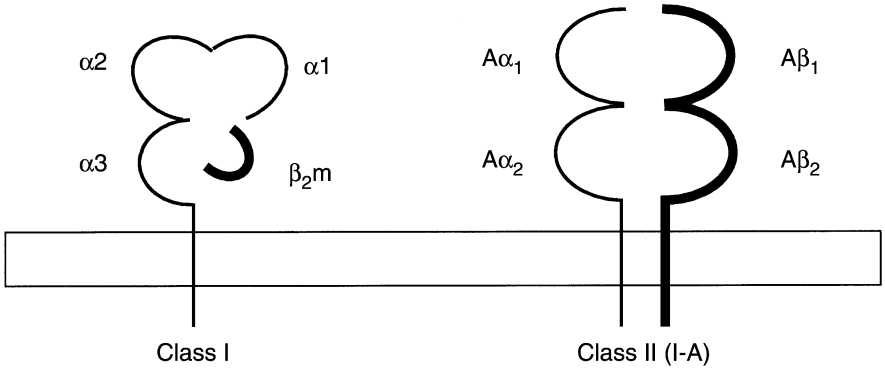

Fig. 1. Schematic structure of major histocompatibility complex (MHC) class I and class II antigens. MHC class I molecules are composed of a polymorphic heavy chain composed of three α domains. The heavy chain, which is encoded within the MHC (K, L, or D in the mouse) is noncovalently associated with β_2-microglobulin. The β_2-microglobulin gene lies on chromosome 2 outside the MHC locus. MHC class II antigens are composed of α and β chains, both of which are encoded within the MHC locus.

event leads to T-cell activation and the generation of a vigorous immune response in which donor MHC proteins become targets for cellular and humoral effector responses leading to graft injury and destruction. Among the first knockout mice that were produced were animals in which the genes encoding components of MHC antigens had been disrupted (Fig. 1). MHC class I-deficient mice were produced through targeted disruption of the β_2-microglobulin gene [9, 10]. This gene is located outside of the MHC, and it encodes the nonpolymorphic component of the class I heterodimer. Because β_2-microglobulin is required for the normal assembly and cell-surface expression of the class I antigen complex, β_2-microglobulin–deficient mice were grossly deficient in their expression of all MHC class I antigens. As the normal development of $CD8^+$ T cells requires a productive interaction between developing thymocytes and MHC class I antigens in the thymus (positive selection), β_2-microglobulin–deficient mice are also grossly deficient in $CD8^+$ T cells [9, 10]. This observation proved the critical requirement of class I antigens in the positive selection of $CD8^+$ T cells. Class II-deficient mice were produced by creating a null mutation in the $A\beta^b$ chain of the I-A molecule. In these mice, which are of the $H\text{-}2^b$ genetic background, I-A is the only class II protein that is expressed [11]. Thus, the destruction of the $A\beta^b$ gene completely ablates class II antigen expression in these animals. As MHC class II antigens are required for the positive selection of $CD4^+$ T cells, the class II-deficient mice were grossly deficient in their complement of $CD4^+$ T cells [12, 13].

Because the β_2-microglobulin and $A\beta^b$ genes are located on different chromosomes, these mutations could be combined by simple breeding, and mice that were deficient in the expression of both classes of MHC antigens were generated. It was hoped that this approach might yield "universal donors" that could be transplanted across MHC disparities without inducing rejection. From this perspective, the initial experiments with skin grafts were somewhat surprising and disappointing. Skin grafts from class I-deficient mice transplanted across major and most minor MHC disparities were rapidly rejected [14]. Similarly, vigorous rejection of class II-deficient skin grafts was also observed [15–17]. Remarkably, skin grafts devoid of both class I and class II were also rejected rapidly [16, 18] through a T-cell–dependent mechanism.

Earlier cell culture studies had shown that D^b class I heavy chains could be detected on the surface of mutant cell lines that lacked β_2-microglobulin [19]. When β_2-microglobulin–deficient mice were produced, class I D^b heavy chains could also be detected on the surfaces of their lymphocytes [10, 20]. Moreover, using stimulator cells from β_2-microglobulin–deficient mice, it was demonstrated that these class I heavy chains could prime cytotoxic T-cell responses [20] even across minor histocompatibility differences [21], suggesting that they could function to present peptides to T cells. The presence of class I heavy chains on the surface of thymic epithelium was also sufficient to mediate positive selection of small numbers of $CD8^+$ T cells in β_2-microglobulin–deficient mice [22, 23]. Thus, in β_2-microglobulin–deficient mice, class I antigen expression is markedly reduced, but not completely absent, and this "leaky" phenotype may provide an explanation for the rejection of combined class I^-/II^- skin grafts. Indeed, studies by Lee et al showed that rejection of MHC-deficient skin was mediated by $CD8^+$ T cells that recognize free class I heavy chains [24]. Accordingly, more effective strategies to eliminate class I expression, such as targeted disruption of genes encoding individual class I heavy chains [25] or transcription factors that regulate MHC expression [26], may provide a more complete protection against rejection.

Compared with the results with skin grafts, the survival of vascularized organ grafts that lack MHC antigens was generally prolonged. This probably reflects basic differences in the character and requirements for immune responses to directly vascularized organ grafts compared with skin. For example, prolonged survival has been documented for donor class I^- [27, 28], class II^- [29], or

combined class I^-/II^- cardiac allografts [29]. The survival of class I^- liver allografts is also prolonged, even when the recipient is presensitized [28]. However, in most circumstances, the MHC-deficient allografts are eventually rejected.

In kidney allografts, we found that the glomerular filtration rate (GFR) is preserved in allografts from donors that are deficient in class I [30], class II (abstract; Mannon et al, *J Am Soc Nephrol* 5:984, 1994), or both class I and II antigens (abstract; Mannon et al, *J Am Soc Nephrol* 8:660A–661A, 1997). In the early period after transplantation, the degree of preservation of GFR was similar whether the graft lacked a single or both classes of MHC antigens. Despite the improvement in GFR compared with controls, the histomorphology of the MHC-deficient kidney allografts is markedly abnormal and is characterized by substantial inflammatory cell infiltrates with tubulitis and vasculitis. However, there are some differences in the character of the intragraft immune response in MHC-deficient grafts compared with controls. For example, a reduced tempo for the accumulation of $CD8^+$ T cells in the graft is seen in the class I-deficient transplants [31], whereas a dramatic reduction in the number of $CD4^+$ T cells is seen in class II^- kidney allografts compared with controls (abstract; Mannon et al, *J Am Soc Nephrol* 5:984, 1994). In addition, antibodies against donor class I antigens are not detected in serum of recipients of class I^- [30] or class I^-/II^- kidneys (abstract; Mannon et al, *J Am Soc Nephrol* 8:660A–661A, 1997) compared with the elevated levels that are seen in controls. Thus, reduced levels of expression of donor MHC antigens on kidney allografts ameliorate kidney injury in acute rejection and can reduce the tempo of accumulation of T cells in the graft. However, even in the absence of a normal expression of class I and II proteins, a vigorous intragraft immune response occurs. Although this may be triggered, in part, by the recognition of free class I heavy chains, the intensity of the reaction suggests a role for indirect alloantigen recognition (discussed later in this article) and/or for factors in the microenvironment of the graft that may regulate the dimension and severity of the intragraft immune response independent of cell surface MHC expression.

Although reduced expression of donor alloantigens can ameliorate rejection, the absence of donor MHC antigens can, in some circumstances, be detrimental to graft survival because of lysis of MHC-deficient tissue by natural killer (NK) cells from the host. NK cells are large lymphoid cells that possess receptors for MHC class I proteins [reviewed in 32]. The engagement of these receptors by MHC class I proteins causes inhibition of NK cell function. Thus, the absence MHC antigens on the surface of a cell may preferentially target these cells for lysis by NK cells. The potential for NK cells to mediate the rejection of MHC-deficient allografts was demonstrated in studies by Bix et al [33]. These investigators found that bone marrow transplants from MHC class I-deficient donor mice were rejected in a matter of days, whereas the wild-type marrow survived indefinitely. This robust rejection was shown to be mediated by NK cells. A role for NK cells in rejection of MHC-deficient skin or vascularized organ allografts has not been demonstrated. In our own studies, we can detect only very small numbers of cells bearing NK markers in class I-deficient kidney grafts, and it seems unlikely that they play any substantive role in the rejection of MHC-deficient kidney grafts (Mannon et al, unpublished observations).

Pathways for direct and indirect allorecognition

Rejection is initiated through specific interactions between the T-cell receptor on recipient lymphocytes and MHC antigens derived from the donor. These recognition events serve to activate a specific population of T cells, leading to a vigorous immune response. The recognition of alloantigens may occur through two distinct pathways (Fig. 2) [reviewed in 34]. In the direct recognition pathway (which is unique to alloimmune responses), recipient T cells interact directly with MHC antigens on the surface of donor antigen-presenting cells. In the indirect pathway, recipient T cells recognize peptides derived from donor MHC proteins that have been processed by recipient antigen presenting cells (APCs) and are presented in the context of self MHC [reviewed in 35]. This is the usual pathway used by the immune system for processing and the presentation of nominal antigens.

It has been suggested that these two pathways for alloantigen presentation and recognition may play distinct roles in the pathogenesis of rejection. Accordingly, the direct recognition pathway may be primarily responsible for the intensity and high precursor frequency of alloimmune responses in acute rejection. The high precursor frequency of cells capable of direct recognition of allo-MHC has been attributed to differences in density of cell surface MHC molecules or the through recognition of alloreactive MHC molecules by "molecular mimicry" [reviewed in 34]. On the other hand, the repertoire for recognition of alloantigens through the indirect pathway is much smaller [36, 37]. This indirect pathway may be important in the pathogenesis of chronic rejection [36, 38] and may be more resistant to conventional immunosuppressive agents [39]. Although the relative roles of direct and indirect allorecognition in transplant rejection are difficult to distinguish *in vivo*, the capacity to genetically manipulate MHC gene expression in mice has provided an experimental approach to study the contributions of the distinct allorecognition pathways to graft rejection.

The existence and initial characterization of the indi-

rect pathway for allorecognition were addressed in studies by Auchincloss et al using class II-deficient mice [15]. They found that class II-deficient skin grafts were rapidly rejected by wild-type mice, and they hypothesized that this rejection was mediated by recognition of MHC class I antigens by $CD8^+$ T cells. However, the administration of anti-CD8 antibodies to the recipient did not prevent rejection of class II-deficient grafts. In the animals that received anti-CD8 antibody, a small population of residual $CD8^+$ cells could be detected, and these cells seemed to be responsible for the rejection of the class II-deficient allografts [17]. Furthermore, the cytolytic activity of these cells required help from $CD4^+$ T cells that had been sensitized to donor-derived peptides through the indirect pathway for allorecognition. These experiments provided clear-cut evidence for the function of the indirect allorecognition pathway in mediating allograft rejection *in vivo*. Additional evidence for indirect allorecognition in skin graft rejection was demonstrated by these authors using class I^-/II^- mice. As discussed earlier in this article, wild-type mice efficiently reject skin allografts from class I^-/II^- donors [18]. In order to examine the role of the indirect allorecognition pathway in the rejection of MHC-deficient grafts, class II-deficient mice were bred with transgenic mice that express class II antigens only on thymic medullary epithelium [40]. The resulting mice have normal numbers of $CD4^+$ T cells but lack class II antigens on their APCs; therefore, they are unable to present donor alloantigens through the indirect pathway. These class II knockout/I-E transgenic mice do not reject MHC-deficient skin grafts [24], suggesting that the rejection of MHC-deficient skin was accomplished through indirect allorecognition.

Relative roles of $CD4^+$ and $CD8^+$ T cells in transplant rejection

The $CD4^+$ and $CD8^+$ populations of T cells specifically recognize antigen in the context of MHC class II or class I, respectively, and properties of the CD4 and CD8 proteins are critical for these specific MHC interactions [41]. These cell populations also serve different functions in allograft rejection. Although their roles are not exclusive, the $CD4^+$ population tends to exert "helper" functions in coordinating the alloimmune response, whereas $CD8^+$ cells comprise the major effector population of cytolytic cells. The relative requirements for $CD4^+$ and $CD8^+$ T cells during allograft rejection have been studied extensively, and roles for both subpopulations are supported by past experiments [reviewed in 42]. For example, in the setting of an isolated MHC class I difference between donor and recipient, rejection depends predominantly on the $CD8^+$ T-cell response, as demonstrated by adoptive transfer studies in which purified $CD8^+$ T cells caused rejection [43] or in studies in which the depletion of $CD8^+$ T cells abrogated rejection [44]. On the other hand, depleting recipients of $CD4^+$ T cells with antibodies results in prolonged allograft survival [45, 46] and, in some cases, donor-specific tolerance [47, 48]. The interpretation of this work is complicated, however, by limitations of the experimental approaches that were used [reviewed in 49]. Although depleting antibody regimens can be used effectively to reduce a particular cell population, subpopulations of the target cells that retain functional reactivity may persist [50]. Direct stimulation of target cells by the antibody may be an additional confounding factor. Finally, in adoptive transfer studies, it may be difficult to completely eliminate contamination of T-cell subpopulations that are purified by negative selection.

The generation of mice with targeted disruptions of the CD4 or CD8 genes has proved useful in further characterizing the cellular requirements for graft rejection without some of the problems inherent to antibody depletion or adoptive transfer studies. To address the requirement for $CD8^+$ T cells during graft rejection, CD8-deficient ($CD8-/-$) mice were transplanted with MHC-disparate skin [51–53] and cardiac allografts [53]. The CD8-deficient mouse lines lack $CD8^+$ T cells and exhibit minimal cytotoxic T-lymphocyte (CTL) function against MHC class I [54]. Despite the lack of CTL function, both skin and heart allografts were rejected with the same tempo in $CD8-/-$ mice as in wild-type recipients [52, 53]. Allografts were also rejected efficiently by mice lacking perforin [55–57] or granzyme B [58]. These molecules serve as the molecular effectors for cytolytic activity of $CD8^+$ T cells [59]. Thus, experiments using knockout mice suggest that $CD8^+$ T cells and the molecules that mediate their cytotoxic effects are not absolutely required for allograft rejection.

CD4 knockout mice have been used in a similar fashion to address the role of $CD4^+$ T cells in rejection. $CD4^+$ T cells are absent in CD4-deficient ($CD4-/-$) mice, whereas the $CD8^+$ T-cell population appears to be normal; T-helper responses in $CD4-/-$ mice are also markedly reduced [60]. The survival of skin or cardiac allografts that are transplanted into $CD4-/-$ mice is prolonged compared with controls [53]. When $CD4-/-$ mice are reconstituted with purified $CD4^+$ T cells, rejection responses proceed as in wild-type mice. These studies further demonstrate the important role of $CD4^+$ T cells in allograft rejection.

Role of cytokines in allograft rejection

When T cells are activated following alloantigen recognition, the alloimmune response is amplified and shaped through the actions of soluble cytokines, which promote T-cell growth and differentiation [reviewed in 61]. There are several lines of evidence supporting a key role for cytokines in the pathogenesis of rejection. For example, the expression of a wide range of cytokines is

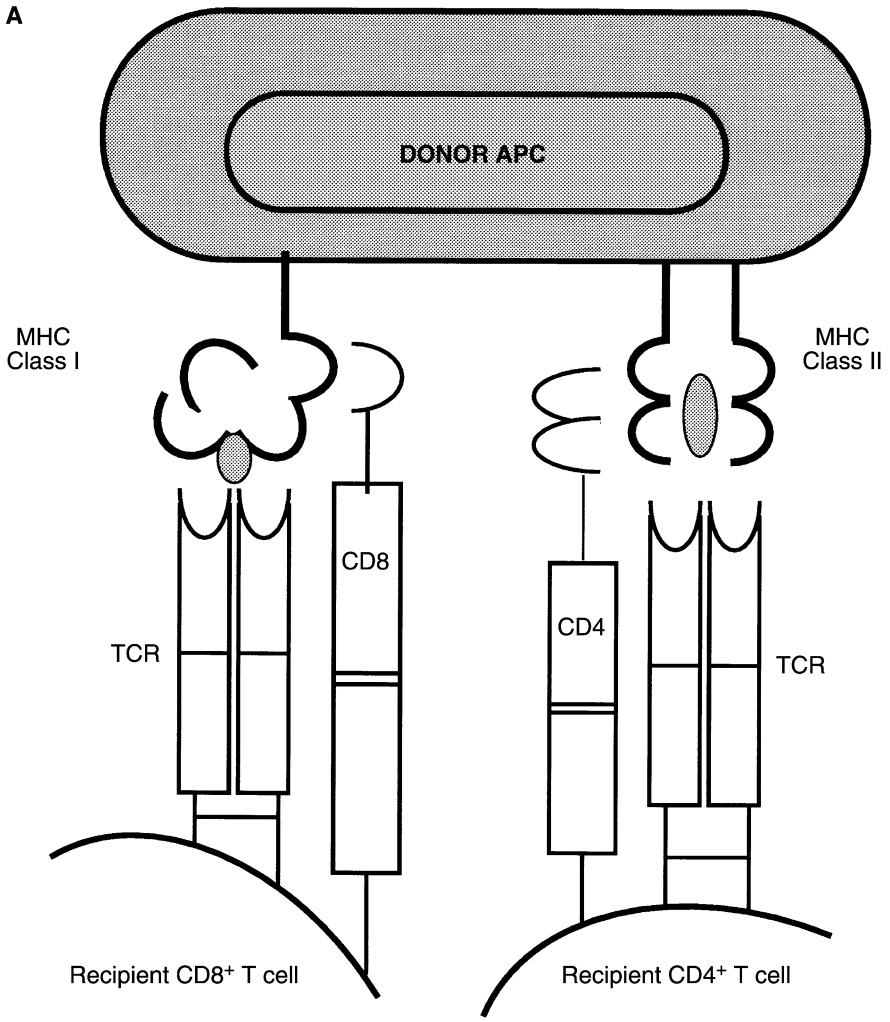

Fig. 2. Pathways of recognition of foreign antigens. (*A*) Direct allorecognition. T cell receptors (TCR) on recipient T cells interact directly with intact donor major histocompatibility complex (MHC) antigens on antigen presenting cells (APCs) derived from the donor organ (shaded circles). $CD8^+$ T cells preferentially interact with MHC class I antigens, whereas $CD4^+$ T cells recognize MHC class II antigens. (*B*) Indirect allorecognition. Recipient T cells recognize peptides derived from donor MHC proteins that have been processed by recipient APCs and are presented in the context of self MHC expressed on APCs from the recipient.

up-regulated within rejecting allografts compared with nonrejecting isografts [62]. Moreover, the putative mechanism of action of cyclosporine and FK-506 in suppressing rejection is thought to be their potent ability to inhibit the transcription of cytokine genes [reviewed in 63]. However, it has been difficult to ascribe specific functional roles for individual cytokines in allograft rejection.

In addition to the effects of cytokines to promote T-cell proliferation, specific profiles of cytokine production have been correlated with distinct T-cell functions. Such responses have been suggested to play a key role in a variety of immune responses [reviewed in 64], including allograft rejection [reviewed in 65]. For $CD4^+$ cells, which are the central regulatory cell in allograft rejection (discussed earlier in this article), two major functional phenotypes have been described that are defined by specific patterns of cytokine production (Table 1). The Th1 phenotype is characterized by production of cytokines such as interleukin-2 (IL-2), interferon-γ (IFN-γ), and lymphotoxin. Th1 cells generally function to promote cellular immune responses. In contrast, the Th2 phenotype is characterized by the production of IL-4, IL-5, IL-6, and IL-10 and is associated with the development of humoral immune responses, in particular those that involve production of IgE.

Following transplantation, it has been suggested that a predominant Th1 response will promote rejection, whereas a Th2 response will favor the development of tolerance [reviewed in 66]. This hypothesis has been based primarily on circumstantial evidence showing predominant expression of Th1-type cytokines in rejecting grafts [62, 67, 68] and expression of Th2 cytokines within allografts in tolerant hosts [69–71]. It has been difficult, however, to directly test the functional relevance of this Th1/Th2 paradigm in allograft rejection. Recently, mice with targeted disruptions of cytokine genes have been used to examine the roles of individual cytokines in allograft rejection and to evaluate critically the relationship of Th1 and Th2 responses in the development of rejection and allograft tolerance.

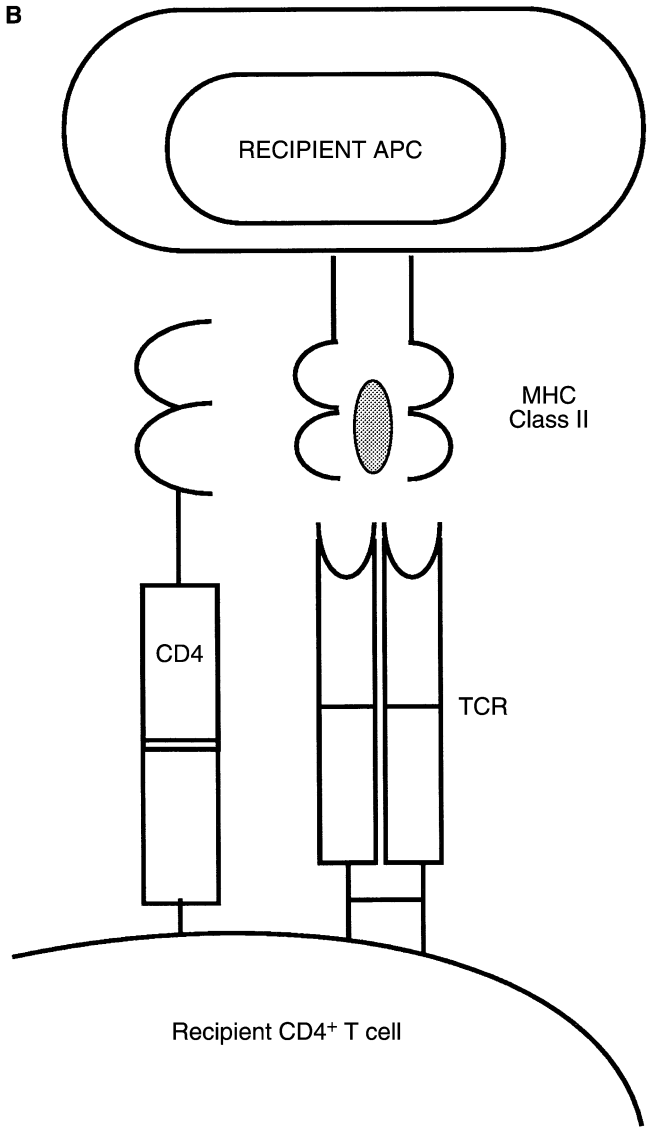

Fig. 2. Continued.

Interleukin-2 is a Th1 cytokine that has been considered to have fundamental importance in the pathogenesis of transplant rejection. The inhibition of IL-2 production has been suggested to underlie the efficacy of cyclosporine and FK-506, two pharmacological agents that are extremely effective in preventing allograft rejection [63]. Thus, it was quite surprising when Steiger et al reported that islet allografts were rapidly rejected by IL-2 knockout mice and that the character and histological pattern of rejection in IL-2–deficient mice were virtually identical to controls [72]. However, despite their ability to reject islet transplants, IL-2–deficient mice failed to generate CTL activity *in vitro*, and their responses to mitogens were impaired unless exogenous IL-2, IL-4, or IL-7 were provided [72]. Expression of IL-15, another potent T-cell growth factor, was enhanced in allografts transplanted into IL-2–deficient allograft recipients [73], suggesting that redundancy within the cytokine network might be sufficient to support rejection when IL-2 is absent.

Interferon-γ is another Th1 cytokine that was considered essential for allograft rejection [62]. IFN-γ activates T cells and macrophages and has potent effects on MHC antigen expression [74]. By virtue of its effects to promote T-cell differentiation toward the Th1 phenotype [64], enhanced IFN-γ production would be anticipated to promote the development of cellular immune responses that would lead to graft rejection. As with the IL-2–deficient mice, transplantation experiments using mice with targeted disruption of the IFN-γ gene yielded unexpected results. Allografts transplanted into IFN-γ–deficient recipients were rejected with a tempo and severity equivalent to allografts in wild-type mice [75, 76]. Moreover, mRNAs for the Th2 cytokines IL-4 and IL-10 were expressed at high levels in IFN-γ–deficient recipients [75]. These results clearly demonstrated that IFN-γ is not necessary for acute allograft rejection. Taken together with results from the IL-2 knockout experiments, it is clear that rejection can proceed in the absence of prototypical Th1-type cytokines. In this setting, predominant expression of Th2 cytokines is not sufficient to prolong graft survival.

After experiments with knockout mice demonstrated that Th1 cytokines were not necessary for allograft rejection, the converse experiments were done to determine whether Th2 cytokines were required to induce allograft tolerance. In these experiments, mice lacking IL-4 were used. IL-4 is required for the normal development and differentiation of Th2 cells, and IL-4–deficient mice are unable to mount Th2 immune responses [77]. Islet [78] and cardiac allografts [79] were rapidly rejected in IL-4–deficient mice. In wild-type mice, blocking the B7-CD28 and CD40-CD40 ligand pathways for T-cell costimulation leads to long-term allograft survival and, in some cases, donor-specific tolerance [80]. This treatment also effectively induces long-term survival of islet [78] and cardiac allografts [79] transplanted into IL-4 −/− mice. Thus, prolonged allograft survival can be induced in the absence of IL-4, and immune deviation toward a Th2 pattern is not required to achieve long-term graft survival in these animals.

More recent experiments by Lakkis et al have examined whether or not the absence of Th1 cytokines facilitates the development of allograft tolerance [79]. In these studies, costimulatory blockade with CTLA4-Ig and antigp39 antibodies induced long-term survival of cardiac allografts in wild-type mice, but not in IFN-γ (−/−) recipients [81]. The treatment with neutralizing anti–IFN-γ antibodies also abrogated the ability of costimulatory blockade to improve graft survival. Thus, these experiments using knockout mice clearly show that the

Table 1. Principal subsets of mouse $CD4^+$ T cells

	Th1	Th2
Cytokines secreted	IL-2, IFN-γ, lymphotoxin	IL-4, -5, -6, -9, -10
	TNF-α, GM-CSF, IL-3	TNF-α, GM-CSF, IL-3
Immune functions	Macrophage activation	B cell activation
	Delayed-type hypersensitivity	Mast cell and eosinophil production
	Antibody dependent cell-mediated cytotoxicity	Antibody production
Transplant response	??Promotes rejection	??Supports tolerance

absence of a quintessential Th1 cytokine does not facilitate long-term graft survival. Furthermore, they have suggested an unexpected requirement of IFN-γ for the development of allograft tolerance.

In summary, studies using cytokine-knockout mice suggest that the pathogenesis of allograft rejection and tolerance cannot be strictly explained through the Th1/Th2 paradigm. The complexity of the response is further illustrated by studies of cytokine expression in human transplant biopsies showing simultaneous expression of both Th1 and Th2 cytokines in rejecting grafts [82, 83].

Issues in the interpretation of gene targeting experiments

During the course of this review, we have emphasized the many positive attributes of gene targeting as a tool for studying transplantation biology. However, as with any other approach, there are factors that need to be considered when interpreting experiments that employ this technology. One is the potential influence of background genes on phenotype. Most knockout mice are produced using embryonic stem cells derived from mice of the 129 strain. Because 129 mice are poor breeders with low fecundity, chimeras and their progeny bearing the targeted mutation are often bred and expanded using other inbred lines, such as C57BL/6. Mice that are generated in this way will possess a random and heterogeneous mix of background genes. In transplantation experiments, these background genes will determine the array of major and minor histocompatibility antigens that are expressed and may influence the character of the recipient's immune responses. These potentially confounding effects can be minimized by using mice that bear the mutation on inbred backgrounds. Such animals can be obtained through repeated backcrossing that may require 12 to 18 months to generate. Alternatively, the mutation can be maintained on a 129 background from the beginning stages of breeding, keeping in mind that this will increase the time necessary to generate adequate numbers of animals for experiments. As an additional solution to this dilemma, several new embryonic stem cell lines derived from non-129 inbred mouse lines have been developed recently [84, 85].

When performing studies with knockout mice, it is also important to distinguish physiological events that are due to the absence of the targeted gene product from developmental defects or compensatory effects related to the absence of the gene product through embryogenesis. For example, it has been suggested that the rejection of allografts in IL-2–deficient mice may be mediated by up-regulation of other cytokine genes, such as IL-15 [73]. These compensatory effects might not occur in the normal immune system, and therefore, these studies may underestimate the contribution of IL-2 in allograft rejection. Although an absolute distinction between these possibilities may be quite difficult to achieve, combining gene targeting with other experimental interventions can be helpful. This approach has been used by Lakkis et al in their studies exploring the role of IFN-γ in tolerance induction (discussed earlier in this article) [79, 81]. These investigators found that the genetic absence of IFN-γ and neutralizing antibodies against IFN-γ had similar effects in interfering with the ability of costimulatory blockade to induce tolerance. Similar results using two complementary interventions provide a compelling argument that the inability to induce tolerance is due to the absence of IFN-γ and not due to generalized abnormalities in the immune system caused by the absence of IFN-γ during fetal development.

Many of the experiments using gene targeting to study transplantation have used homozygous knockout animals, with complete deficiencies of the target gene. This provides an attractive experimental approach for understanding the role of a particular gene product in rejection. In the human population, there may be genetic variations that incrementally alter the function of a gene. For example, polymorphisms in the genes for the cytokines tumor necrosis factor-α and IL-10 may alter the production of these cytokines, and these polymorphisms have been suggested to affect the character of allograft rejection [86]. The effects of this type of mutation on graft rejection could also be explored, using, for example, mice that are heterozygous (+/−) for a targeted mutation. These animals often exhibit approximately an 50% reduction in total expression of the target gene. Alternatively, in animals with duplication of the target gene, the role of enhanced expression of a particular gene product might be examined.

CONCLUSION

In this article, we reviewed some of the applications of gene targeting to studies of the immunobiology of transplantation. We have focused on studies that provide an illustration of the potential contributions of this technique to understanding the pathogenesis of allograft rejection. In these discussions, we have attempted to highlight the unique advantages of gene targeting and its contribution to unraveling the pathogenesis of allograft rejection. Although we have concentrated on the use of these genetic techniques as research tools, one might ask whether there is a potential for these approaches to be applied in the clinic. For the moment, the answer to this question is "no." Isolation of embryonic stem cells that are capable of contributing to the germline in reconstituted blastocysts has so far only been accomplished in mice. Furthermore, even if the problem of deriving germline competent embryonic stem cells from larger animals can be solved, such an approach would only be applicable to nonhuman donors. Because of the large number of ethical and practical hurdles that must be cleared before xenotransplantation can be considered for clinical use [87], along with the technical problems of embryonic stem cell biology in large animals, practical applications of this technology could be considered in only the distant future.

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